International Journal of Biological Research and Development (IJBRD)
ISSN (P): 2250-0022; ISSN (E): Applied
Vol. 8, Issue 1, Dec 2018, 17-24

ANTIBACTERIAL ACTIVITY AND ANTIOXIDANT ACTIVITY OF *CARICA PAPAYA* ON SOME ENTERIC BACTERIAL ISOLATES OF PUBLIC HEALTH IMPORTANCE

R. K. SUMATHI[1] & SINDOOJA. S[2]

[1]*Associate Professor, Department of Microbiology, Sri Ramakrishna College of Arts and Science for Women, Tamil Nadu, India*

[2]*PG Student, Department of Microbiology, Sri Ramakrishna College of Arts and Science for Women, Tamil Nadu, India*

ABSTRACT

In the present study, ethanol extract and aqueous extract of Carica papaya in different concentration were evaluated for the antimicrobial properties against Enteric pathogenic organisms such as Escherichia coli, Shigella sp., Klebsiella sp., and Pseudomonas sp., by using agar well diffusion method. The extracts were screened for the phytochemical constituents using standard procedure. The anti-oxidant activity of ethanol and aqueous extract fractions from the leaf of papaya were evaluated and ethanol extract showed the strong DPPH activity, Nitric oxide radical scavenging assay, Ferric Reducing/Antioxidant Power (FRAP) Assay and Hydrogen Peroxide Scavenging Activity (H_2O_2). The leaves of papaya and these compounds might be used as natural antioxidants. Antibacterial activity against the test isolates was an indication of the possibility of sourcing alternative antibiotic substances in these plants for the production of newer antibacterial agents.

KEYWORDS: Antibacterial Activities, Carica Papaya, Antioxidant & Enteric Bacteria

Received: Jul 11, 2018; **Accepted:** Aug 01, 2018; **Published:** Oct 10, 2018; **Paper Id.:** IJBRDDEC20183

Original Article

INTRODUCTION

According to WHO, around 21,000 plant species have the potential for being used as medicinal plants. Many studies have been conducted to evaluate the biological activities of various parts of *Carica papaya*. The two important compounds are chymopapain and papain, which aid in digestion. The fruit and seed of *Carica papaya* have showed bacteriostatic activity against several enteropathogens in human. Phytochemical studies on the leaves of *Carica papaya* shows the presence of various compounds, including piperidine alkaloids such as carpaine, pseudocarpaine, and dehydrocarpaines I and II. Recently carpaine, quinic acid, six malic acid derivatives and four flavonoid glycosides in the leaf extract was identified. *Carica papaya* Linn. (Family: Caricaceae) primarily cultivated for its fruit and the young leaves are consumed as a vegetable. Different parts of the plant have been used in traditional medicine to treat various diseases. The principle for inhibiting the disease causing organism is by binding their protein molecules, acting as chelating agents (selective binding polyvalent metal ions so that the latter loses its biological activities), altering their biochemical systems, preventing the utilization of available interest to the microorganism and other causes inflammation analysis of microbial cells (Garrod *et al*., 1995). However, a very important step in the screening of the sanitizing and preservative activity of a plant material was to evaluate its antimicrobial properties. The importance of evaluation of the antimicrobial

properties of *Carica papaya* leaves on some selected microorganisms and also to verify its minimum inhibitory concentration. The active components in this trait are expected to be unfriendly to the growth of at least some microorganisms, especially the disease causing, such as *Escherichia coli*, *Pseudomonas* species, *Klebsiella* species and *Shigella* species. Therefore, in this study the antimicrobial and antioxidant properties of the leaf of *Carica papaya* will be discussed.

MATERIALS AND METHODS

Collection of Plant Materials

The young leaves of *Carica papaya* are used in the research. Which were collected from the local area of Coimbatore. Identification and authentication of the plant material have been done at a Herbarium unit in the Botanical survey of India, Coimbatore with the following voucher numbers 1877. The samples were then washed and rinsed with distilled water. Samples are air-dried for 2-3 weeks. The dried leaves were made into powder by using mortar and pestle. Maceration method was described by Mukhtar and Tukur (1999). The powder was collected and stored in zip-lock polythene air-tight bag.

Preparation of Plant Material

The leaf of *Carica papaya* was separately extracted with hot water and 95% ethanol. These were prepared using the method as described by Oyagae *et al*., 1999.

Test Organisms

Clinical isolates of *Escherichia coli, Pseudomonas sp, Kelbsiella sp,* and *Shigella sp,* were obtained from the Department of Microbiology of Sri Ramakrishna Hospital, Coimbatore. Identification of the isolates was done by standard microbiological procedures (Cappuccino, 2014). The antimicrobial potency of the leaf extract was evaluated using the four bacterial strains causing food poisoning disease. The potential effectiveness of the extract can be used as a natural alternative to control food poisoning.

Preparation of Plant Extract

The ethanol and aqueous extract of plant sample was carried out by suspending 25grams of the finely ground leaves in 150 milliliter of distilled water and 95% ethanol. The hot water extraction was done at 80°C in soxhlet apparatus for 1/2hours. The ethanolic extraction was then filtered through a Whatman filter paper. The filtered extract was then sterilized using a membrane filter and were stored in the refrigerator at 4°c until used (Omojosola and Awe, 2004).

Estimation of Antioxidant Activity

DPPH Radical Scavenging Activity

The free radical scavenging activity of ethanolic and aqueous extract of the plant extract was measured by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH). The scavenging activity for DPPH free radicals was measured according to the procedure described by (Braca *et al*., 2001). An aliquot of 3 ml of 0.004% DPPH solution in methanol and 0.5 to 2.5µl of plant extract/ascorbic acid at various concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. Decolorization of DPPH was determined by measuring the absorbance at 517nm. A control was prepared using 0.1ml of the respective vehicle in the place of plant extract/ascorbic acid. The percentage inhibition of DPPH radicals by the extract/compound was determined by comparing the absorbance values

of the control and the experimental tubes.

Nitric Oxide Radical Scavenging Assay (Garrat., 1964)

The determination of Nitric oxide scavenging activity of the sample was determined byadding 400µL of 100mm sodium nitroprusside, 100µl of Phosphate buffered saline (pH - 7.4) and 100µl of different concentration of plant extracts. This reaction mixture was kept for incubation at 25°C for 150 minutes. To 0.5 ml of the above solution, 0.5 ml of Griess reagent was added (0.1ml of sulfanilic acid and 200µl naphthylethylenediamine dichloride (0.1%) w/v)) and kept on incubation at room temperature for 30 minutes, and finally absorbance is observed at 540nm. All the reactions were performed in triplicates, and their percentage inhibition was calculated by the following formula where 'A' stands for adsorbents:

Inhibition percentage of nitric oxide= [(Acontrol-A sample) /Acontrol)] ×100

Hydrogen Peroxide Scavenging Activity (H_2O_2)

The ability of the extract to scavange hydrogen peroxide was resoluted according to a standard method by Ruch *et al.*,(1989). The extract 0.1ml was added to 4ml of phosphate buffer and mixed with 600µl of 400mm H_2O_2. The ability of the extracts to scavenge hydrogen peroxide was determined according to a standard method by Ruch *et al*., Plant extract 0.1ml was added to 4ml of phosphate buffer and mixed with 600µl of 40mm H_2O_2. Hydrogen peroxide concentration was resolved spectro photometrically at 230 nm in the absence and presence of the extract. A control was kept without the extract. For each concentrate a different clear containing the concentrate in support arrangement without hydrogen peroxide was utilized. Level of hydrogen peroxide searching (I) was figured as

Inhibition of Hydrogen peroxide = (Abs control – Abs test)/Abs control X 1

Ferric Reducing/Antioxidant Power (FRAP) Assay

The antioxidant capacity of plant extract samples was estimated according to the procedure described by Benzie and Strain (1996). FRAP reagent (900µl), prepared freshly and incubated at 37ºC, was mixed with 90µl of distilled water and 30µl of the test sample, or acetone (for the reagent blank). The test samples and reagent blank were incubated at 37ºC for 30 minutes in a water bath. The FRAP reagent contained 2.5ml of 20mmol/l TPTZ solution (2, 4, 6-Tripyridyltriazine) in 40mmol/l HCl plus 2.5 ml of 20mmol/l FeCl3.6H2O and 25ml of 0.3mol/l acetate buffer, pH 3.6 (Benzie and Strain, 1996). At the end of the incubation period the absorbance readings were recorded immediately at 593 nm using a spectrophotometer. The known Fe (II) concentration ranging between 100 and 2000µmol/l (FeSO4.7H2O) was used for the preparation of the calibration curve. The parameter Equivalent Concentration (EC1) was defined as the concentration of antioxidant has a ferric- TPTZ reducing ability equivalent to that of 1mmol/l FeSO4.7H2O. EC1 was calculated as the concentration of antioxidant giving an absorbance increase in the FRAP assay equivalent to the theoretical absorbance value of a 1mmol/l concentration of Fe (II) solution determined using the corresponding regression equation.

Percentage of inhibition of ferric oxide = Acontrol – Atest / Acontrol X 100

Antibacterial Activity

To perform an antimicrobial activity using various bacterial and fungal species was selected viz., *Escherichia coli, Pseudomonas species, Shigella species, Klebsiella species; Proteus species* are bacterial cultures.

Media and Culture Condition

Muller-Hinton Agar (MHA), Nutrient Broth (NB) and Luria Britani (LB) were used throughout the study for determining the antibacterial assay. The media were adjusted to the pH

Well Diffusion Method

The antibacterial activity and antifungal activity of crude extract extracts was determined by the Well Diffusion method (Bauer *et al*., 1996). MHA plates were prepared by pouring 20ml of molten media into sterile petri plates. After solidification of media, 20-25µl suspension of bacterial inoculums was swabbed uniformly. The sterile paper discs were dipped into required solvents then placed in agar plates. Then 10-50µl of plant extract was poured into the wells. After that, the plates were incubated at 37°C for 24 hours. The assay was carried into triplicates and control plates were also maintained. Zone of inhibition was measured from the edge of the well to the zone in mm. The tested cell suspension was spread on a Muller Hinton agar plate and potato dextrose agar. Well were put into the agar medium using sterile forceps. Plant extract was poured into wells. Then the plates were incubated at 37°C for about 24 hours and control was also maintained. Zone of inhibition was measured from the clear zone in mm.

RESULTS AND DISCUSSIONS

Estimation of Anti-Oxidant Activity

DPPH Radical Scavenging Activity

Aqueous and ethanol extracts exhibit scavenging effects when sample concentration is increased. DPPH is a nitrogen - centered free radical. Antioxidants react with DPPH and convert it to a diphenyl-β-picryl hydrazine. The degree of discoloration indicates the scavenging potentials of the antioxidants. The DPPH scavenging activity of ethanol and aqueous was compared with the activity of the standard. At 500µg/ml concentration, the scavenging activity of the standard Ascorbic acid was 65%, while that of the ethanol and aqueous leaf extract was found to be 79% and 74.20% respectively as reported in Tables1. Thus the scavenging activity of the plant extract was more effective than the standard. Phytochemicals especially in plant phenolic constitute a major group of component that act as primary antioxidants (Hatano *et al*., 1989).

Table 1: DPPH Radicals Scavenging Activity of *Carica Papaya* Extracts

S. No	Concentration	Ethanol Extract	Aqueous Extract	Standard (Ascorbic Acid)
1	100µl	32%	37.65%	25%
2	200 µl	53%	43.36%	50%
3	300 µl	67%	55.32%	60%
4	400 µl	76%	68%	62%
5	500 µl	79%	74.20%	65%

Nitric Oxide Radical Scavenging Assay

The Nitric oxide radical scavenging activity of aqueous and ethanol extracts increased with an increase in concentration of the extracts. At 500µg/ml concentration, the scavenging activity of the standard Ascorbic acid was 87%, while that of the ethanol and aqueous leaf extract was found to be 74% and 77.05% respectively as reported in the Tables2. Thus the scavenging activity of the plant extract was more effective than the standard. Nitric oxide is a free radical created in cells, associated with physiological process, including neurotransmission, antimicrobial and antitumor exercises. But, overproduction of NO can trigger tissue harm and is related to atherosclerosis and hypertension. Phenolic mixes is

predominantly because of their redox properties, which can assume vital part in engrossing and killing free radicals (Hepziba *et al*., 2010).

Table 2: Nitric Oxide Radical Scavenging Assay of *Carica Papaya* Extracts

S. No	Concentration	Ethanol Extract	Aqueous Extract	Standard (Ascorbic Acid)
1	100µl	32.76%	27.38%	26%
2	200µl	34.53%	36.00%	47%
3	300µl	55.05%	51.00%	52%
4	400µl	61.30%	73.00%	69%
5	500µl	74.00%	77.05%	87%

Hydrogen Peroxide Scavenging Activity (H_2O_2)

Ethanolic extract is maximum than in the aqueous extract was found to be 93% and 72% respectively, and H_2O_2 scavenging activity of standard ascorbic acid was found to be 85%. H_2O_2 is rapidly decomposed into oxygen and water in the body, and this may produce hydroxyl radicals (OH) that can initiate lipid peroxidation and cause DNA damage. The ability of plant extracts to scavenge hydrogen peroxide is determined according to the method of (Ruck *et al*., 1989). Hydrogen peroxide (H_2O_2) scavenging action of accepted antioxidants present in plant extracts has been identified broadly (Rajamanikandan *et al*., 2011).

Table 3: Hydrogen Peroxide Scavenging Activity of *Carica Papaya*

S. No	Concentration	Ethanol Extract	Aqueous Extract	Standard
1	100µl %	45%	36%	22%
2	200µl %	58%	42%	38%
3	300µl %	68%	55%	54%
4	400µl %	79%	67%	69%
5	500µl %	93%	72%	87%

The active chemical constituents of plant extract like essential oils, amino acids, tanins, uric acid etc., are responsible for Hydrogen peroxide (H_2O_2) scavenging activity (Srijayanth *et al*., 1999).

Ferric Reducing/Antioxidant Power (FRAP) Assay

The antioxidant potential of *Carica papaya* leaves, the aqueous and ethanol extract was ascertained from FRAP assay based on their ability to reduce TPTZ-Fe(III) complex to TPTZ-Fe(II). The reducing ability of aqueous and ethanol extract with varying concentration (20 -100µg/ml) was examined and compared with standard ascorbic acid. The maximum scavenging activity was found in the ethanol extract to be 86% and 83%. respectively reported in the Table 4.

Table 4: Ferric Reducing Antioxidant Power of *Carica Papaya* Extract

S. No	Concentration	Ethanol Extract	Aqueous Extract	Standard
1	100µl	21%	22%	33%
2	200µl	30%	28%	44%
3	300µl	69%	52%	52%
4	400µl	82%	81%	71%
5	50µl	87%	84%	83%

The phenolic acid and flavonoids are very much responsible for the ferric reducing ability mainly in many of the plant extract (Maruthamuthu *et al*., 2016).

Antibacterial Activity

The antibacterial activity of aqueous and ethanolic extract was reported in the Table 5. The result showed that *E . coli* sp was susceptible both in aqueous and ethanol extract at 100mg/ml concentration and zone of inhibition was 26mm and 28mm respectively. *Shigella* sp being the most susceptible at 100mg/ml concentration of ethanol extract and zone of inhibition was 30mm. *Klebsiella* sp had added susceptibility at 100mg/ml concentration of aqueous extract and zone of inhibition was 30mm, while the *Pseudomonas* sp., was liable at 100mg/ml concentration of aqueous extract and zone of inhibition was 23mm. The secondary metabolites in a number of plant extracts are mainly responsible for antimicrobial, antifungal and anticancer activity (Compean and Ynalvez, 2014).

Table 5: Antibacterial Activity of Enteric Bacteria Isolates

Test Organism	Ethanol Extract		Aqueous Extract		Control (Chloramphenicol)
	50mg/ml	100mg/ml	50mg/ml	100mg/ml	
E. coli	20mm	26mm	22 mm	28mm	20mm
Shigella	28mm	30mm	25 mm	28mm	32mm
Klebsiella sp.,	10mm	28mm	25 mm	30mm	28mm
Pseudomonas sp.,	20mm	15mm	16 mm	23mm	20mm

CONCLUSIONS

Antioxidant and antimicrobial property of Ethanol extract and Aqueous extract of *Carica papaya* leaves were investigated in this study. They can be considered as a natural source to control the enteric pathogen. During a time of rapidly rising antibiotic resistance, new approaches are necessary to fill antimicrobial drug development. Plants remain a unique and underexploited source of bioactive compounds and botanical research tools can be used to guide for future research efforts. To reduce the side effects of the usage of synthetic drugs, we can use medicinal plants for the treatment of common diseases. However, further studies are needed to obtain purified compounds that may be responsible for the activities observed from the tested leaves.

REFERENCES

1. *Bauer. A. W., Kirby. W. M., Sherris. J. C. and Turck. M. (1996). Antibiotic susceptibility testing by standardized single disc method. Am. J. Clin Pathol., 44: 493-496.*
2. *Benzie, I. F. F., and Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power"; The FRAP assay, Analytical Biochemistry, 239(1): 70-76.*
3. *Bouayed, H., Rammal, A., Dicko, C. and Yonos, R. (2007) Chlorogenic acid, a polyphenol from Prunus domestica (Mirabelle), with coupled anxiolytic and antioxidant effects, J. Neurol. Sci., 262. 77-84*
4. *Braca, A., Tommasi, N. D. and Bari, L. D. (2001). Antioxidant principles from Bauhinia terapotensis. J Nat Prod., 64: 892-895*
5. *Cappuccino. G, and Sherma, N. 2014. Microbiology a laboratory manual. 10th edition.*
6. *Compean, K. L. and Ynalvez, R. A. 2014. Antimicrobial Activity of Plant Secondary Metabolites: A Review. Research Journal of Medicinal Plants, 8: 204-213.*
7. *Garratt, D. C. (1964): The quantitative analysis of Drugs. Volume 3. Chapman and Hall ltd, Japan; 456-458.*
8. *Kareem, K. A., & Ali, W. D. A. In–Vitro Evaluation Of Antibacterial Activity Of Silybum Marianum Seeds Oil, Ethanolic Extract And Antibiotics Against Isolated Bacteria.*

9. *Garrod, L. P., Lambert, H. P. and Gray, F. (1995). Antibiotics and Chemotherapy, 4th Ed, Churchill, Livingstons, Edinburgh, London and New York. 501-512.*

10. *Hatano, T., Edamatsu, R., Hiramatsu, M., Mori A. and Fujita, Y. (1989). Effects of the interaction of tannins with co-existing substances and effects of tannins and related polyphenols on superoxide anion radical and on 1,1-diphenyl-2-picrylhydrazyl radical. Chem. Pharm. Bull. 37:2016–2021.*

11. *Hepsibha, B. T., Sathiya, S. Babu, C. S., Premalakshmi, V and Sekar, T. (2010) In vitro studies of antioxidant and free radical scavenging activities of Azima tetracantha. Lam leaf extract. Indian J Sci Technol; 3:571-577.*

12. *Omojasola, P. F. and Awe, S. (2004). The Antibacterial activity of the leaf extract against some selected microorganisms. Bioscience Research Communication 16 (1).*

13. *Oyelola, O. (2005): Evaluation of the hypoglycemic activity of Treculia Africana Decne (root) in normal and diabetic rats. M. Pharm (Clinical Pharmacy) Dissertation.*

14. *Mukhar, M. D. and Tukur, A. (1999) In-vitro screening for activity of Pistia stratiotes extracts. NISEB J I 1:51-60.*

15. *Rajamanikandan, S., Sindhu, T., Durgapriya, D., Sophia, D., Ragavendran P., Gopalakrishnan, V. K. (2011) Radical scavenging and antioxidant activity of ethanolic extract of Mollugo nudicaulis by in vitro assays. Indian J. Pharm. Educ. Res., 45(4):310–316.*

16. *Ruch, R. J., Cheng, S. J. and Klaunig, J. E. (1989) Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis., 10(6):1003-8.*

17. *Srijayanth, S., Raman, A. and Goodwin, B. l. (1999) A comparative study of the Constituent of Aesculusindica, J Med Food, 2(2), 45-50.*